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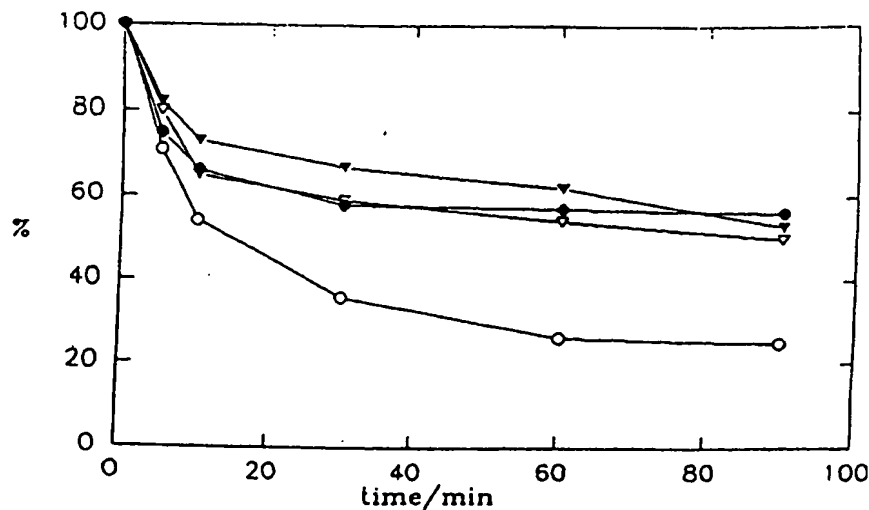
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(54) Title: MEMBRANE AND METHOD FOR THE SEPARATION OF PROTEIN-BOUND SUBSTANCES FROM A PROTEIN-CONTAINING LIQUID BY DIALYSIS

(57) Abstract

The present invention relates to a method of the separation of protein-bound substances (PBS) and, if present, water-soluble substances from a protein-containing liquid (A), e.g. blood or plasma, containing said substances, by dialysis using a special semipermeable membrane and a dialysate liquid (B) by means of a protein having an acceptor function for the PBS from liquid (A). The membrane useful for this method has to provide a tunnel-like porous structure on the liquid (A) side and a port-and adsorption-structure on the dialysate liquid (B) side.

One or both sides of the membrane, i.e. the protein-containing liquid (A) side and the dialysate liquid (B) side of the membrane, are coated with a protein having an acceptor function the PBS. The PBS-containing dialysate liquid (B) obtained after dialysis may then be purified by passing it through a conventional dialyzer in order to remove water-soluble substances and then through a charcoal, and a resin-adsorbent in order to remove PBS from the acceptor protein molecules. The purified dialysate liquid (B) obtained containing the free acceptor protein molecules is then used again as dialysate liquid (B).



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Title

Membrane and method for the separation of protein-bound
20 substances from a protein-containing liquid by dialysis

Description

25 1. Technical Field

The present invention relates to membranes and membrane
transport processes, and in particular to an effective
method for the separation of undesired or potentially
30 harmful protein-bound substances (PBS) and, if present,
water-soluble substances (low-molecular weight and middle-
molecular weight substances) and/or lipophilic substances,
from a protein-containing liquid such as plasma and blood by
dialysis using said membranes.

2. Background

The separation of substances which are strongly bound to a valuable protein from that protein by dialysis is impossible or at least accompanied with a number of difficulties. This is especially relevant if the protein is contained in a complex mixture such as blood or plasma.

In medicine separation techniques are widely accepted for the separation of hydrophilic toxins from blood e.g. in end stage renal disease (ESRD, uremia). Today, maintained hemodialysis (HD) is the live-saving long-term treatment of choice in ESRD patients if no kidney transplantation is available. However, the removal of protein-bound or lipophilic toxins remains, at present, an unsolved problem in medicine. Especially albumin-bound toxins (ABT) have been shown to be involved in the pathogenesis of different exogenous and endogenous intoxications in patients.

Thus ABTs such as butyric, valeric, caproic and caprylic acid, thyroxine and tryptophane, unconjugated bilirubin, mercaptans, digoxin-like immunoreactive substances, benzodiazepine-like substances, aromatic amino acids and phenols seem to be responsible for the induction of hepatic encephalopathy and cerebral edema in fulminant hepatic failure (FHF).

These substances are assumed to be at least partially responsible for lethal cerebral alterations seen in FHF. Moreover, FHF is typically accompanied by coagulopathy, defective ventilation, hypoxemia, pulmonary edema, electrolyte imbalance, acid-base disturbances, renal dysfunction, hypoglycemia, cardiac dysfunction, sepsis, hemorrhage leading to multiorgan failure and death. The mortality has been on an unchanged high level between 60 and

100 % since years, depending on e.g. the age of the patient and the stage of encephalopathy at hospital entry.

In chronic liver insufficiency the same group of ABTs
5 accumulates due to the insufficient metabolism of the liver in the blood and brain although the primary toxins may be different (e.g. ethanol). Therefore, symptoms such as encephalopathy normally seen in FHF may occur in these patients, too, and acute exacerbations mimicking the picture
10 of FHF are seen frequently.

Newborn hyperbilirubinemia is another clinically relevant endogenous intoxication. This form of hyperbilirubinemia is known to have damaging effects to the newborn brain because
15 of the immature blood-brain barrier. Toxic effects of bilirubin in the adult human are under discussion. At least in very high concentrations the toxic effect could be clearly demonstrated.

20 Furthermore, there is a large number of drugs known to have a highly albumin-binding rate in cases of accidental overdosage or suicidal intoxications by e.g. tricyclic antidepressants, digoxin, digitoxin, theophylline or a benzodiazepine.

25 While the importance of ABT in the above-mentioned cases is generally acknowledged, there is also a significant accumulation of albumin-bound toxins in ESRD, e.g. furancarboxylic acids, indoxyl sulfate, aluminum ions,
30 phenols and other organic acids, although patients are on regular HD treatment. The unsatisfactory search for "uremic toxins" among the "middle molecules" spectrum on the one hand and the fact that uremic plasma shows more toxic effects than its ultrafiltrate on the other hand led to the

assumption that some of the "uremic toxins" may be also protein-bound.

Meanwhile this theory which has its origin as early as in
5 1976 has been accepted by the American Society for
Artificial Internal Organs (1993) and many specialists in
the USA, Europe and Japan. This indicates that not only the
liver but also the kidneys take part in the separation of
albumin-bound substances from the blood in order to keep
10 them at non-toxic levels. This is already known for some
drugs and indicator substances (e.g. phenol red) and has
been shown recently also for furan derivatives as a special
excretoric function of the kidneys which cannot adequately
be replaced by hemodialysis. This explains that in dialysis
15 patients the concentrations of these molecules reach levels
which may be of pathophysiological relevance. Underlining
this it has been shown that furancarboxylic acids
accumulating in chronic hemodialysis patients are strong
inhibitors of mitochondrial cell respiration. Furthermore,
20 furan derivatives act as inhibitors of cell proliferation by
interfering with DNA synthesis as shown in vitro.

In vivo relevant long-term complications of maintained HD
treatment in ESRD are seen in almost every patient (e.g.
25 dialysis-associated anemia, suppressed immune response, high
infection rates, encephalopathy) that are caused by
insufficiency of highly specialized or proliferative tissues
(e.g. blood forming tissue). Suggesting a relation between
these in vitro and in vivo results clinical investigations
30 have shown that e.g. the grade of anemia in hemodialyzed
patients strongly correlates with the plasma levels of
furancarboxylic acids.

All states of disease mentioned above have in common not
35 only the ABTs involved in the pathogenesis but also very

high treatment costs for either intensive care and transplantation or long-term treatment with supplementation therapy (e.g. erythropoietin) and repeating hospital stays. Therefore, ABT-associated diseases have a considerable economic dimension with high patient numbers and treatment costs on the one hand and a bad overall prognosis leading to death or invalidity on the other hand (e.g. 400,000 patients on maintained HD worldwide with approximately 60 million treatments per year).

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Treatment of ABT-associated diseases - State of the art

1. Dialysis and Ultrafiltration

15 Because of their affinity to the non-dialyzable albumin molecule ABTs are not able to permeate to a significant degree into the dialysate liquid in vitro and in vivo. Moreover, many of these ABTs are highly lipophilic and not soluble in water.

20

Conventional hemodialysis effectively separates low molecular weight substances (<1500 daltons) from plasma but no protein-bound substances which are believed to be responsible for the detrimental effects of liver insufficiency. Hemodialysis using e.g. large-pore polyacrylonitrile membranes effectively removes middle molecular weight substances (1500-5000 daltons) in addition to the low molecular weight substances but again no protein-bound substances in experimental animals; see De Groot, GH, Schalm SW, Schicht I: Large-pore hemodialytic procedures in pigs with ischemic hepatic necrosis; a randomized study, Hepatogastroenterol. Vol. 31 (1984), 254; De Groot, GH, Schalm SW, Schicht I: Comparison of large-pore membrane hemodialysis and cross-dialysis in acute hepatic

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insufficiency in pigs, EUR. J. Clin. Invest., Vol. 13 (1983), 65.

Therefore, dialysis and ultrafiltration are not satisfactory methods for the separation of these substances.

2. Peritoneal dialysis

Patients undergoing peritoneal dialysis (PD) as an alternative treatment lose up to 10 % of the total serum albumin during a single PD session and by this way, of course, also toxic albumin-bound substances (ABT). In clinical studies typical long-term complications of HD patients, especially the above-mentioned anemia, could not be observed in PD patients. However, this enhanced removal of albumin-bound toxins by PD is accompanied by the loss of albumin which correlates with an increased mortality. Furthermore, PD is associated with a high risk of peritonitis.

3. Erythropoietin therapy

Erythropoietin therapy partially reverses the dialysis/uremia-associated anemia but is expensive. The reason for the anemia is not a lack of endogenous erythropoietin. Arterial hypertension is one of the most often seen adverse effects in this therapy.

4. Kidney transplantation

This method can ameliorate the problem of insufficient removal of ABTs in ESRD. Nevertheless, it has a number of

severe complications including operation risk, intensive immunosuppression, high tumor risk. The overall life expectancy is not different from HD treatment.

5 5. Hemo-/Plasmaperfusion

Hemo- or plasmaperfusion over different or other adsorbents such as ion exchange resins and charcoal are also effective but not sufficiently specific. These methods also remove
10 essential substances like hormones (corticosteroids, thyroxine) which are linked to their own transport proteins (corticosteroid transporting protein or thyroxine transporting protein).

15 In most of the studies made in this regard blood was perfused through sorbent columns containing charcoal or ion exchange resins. By this method protein-bound toxins were removed effectively but this was accompanied by the following disadvantages:

20

- a) loss of blood cells, especially platelets
- b) loss of clotting factors
- c) activation of blood cells (platelets, leukocytes)
- d) activation of the complement system

25 e) loss of essential plasma components blood compounds (hormones, vitamins, growth factors) due to the absolutely unspecific mode of binding
f) release of microparticles, especially when charcoal adsorbents were used.

30

In order to attenuate the adverse effects seen in charcoal perfusion charcoal particles are coated with different blood-compatible substances but these charcoal adsorbents exhibit a decreased ability to bind protein-bound

substances. Hemoperfusion over micro-encapsulated charcoal effectively removes low-molecular weight substances (<1500 daltons) from plasma but no protein-bound substances which are believed to be responsible for the detrimental effects of liver insufficiency.

Of all extracorporeal dialysis and adsorption techniques especially hemoperfusion over an albumin-coated resin (e.g. Amberlite XAD -7) or agarose beads to which albumin had been covalently linked has been shown to effectively remove bilirubin and other toxic protein-bound organic anions from the blood of experimental animals (Wilson RA, Webster KH, Hofmann AF: Toward an artificial liver: in vitro removal of unbound and protein-bound plasma compounds related to hepatic failure, Gastroenterol., Vol. 62 (1972), 1191.; Scharschmidt BF, Plotz PH, Berk PD: Removing substances from blood by affinity chromatography. II. Removing bilirubin from blood of jaundiced rats by hemoperfusion over albumin-conjugated agarose beads, J. Clin. Invest., Vol. 53 (1974), 786. No assessment was made concerning the selectivity or possible adverse effects of this method. However, these albumin-coated adsorbents did not remove low and middle molecular weight substances (Zakim and Boyer (ed.), Hepatology. A textbook of liver disease, W.B. Saunders Company, 1990, p. 479-482).

In conclusion hemo- or plasma-perfusion remain unselective methods.

6. Plasma exchange

In this method the plasma of the patient is separated from blood by filtration or by centrifugation and is replaced by

an albumin solution or plasma. This procedure shows the following disadvantages:

- 5 a) most protein-bound toxins are not only distributed in plasma but also in tissues. After a plasma exchange session a redistribution of the toxins into the purified plasma occurs. This was accompanied with the clinical observation that patients in FHF undergoing this treatment awoke from coma but only for the time of treatment and fell back into
10 deep coma after the end of treatment. Thus, plasma exchange had to be done frequently to be effective, which led to typical immunological reactions mentioned below.
- 15 b) the observed immunological reactions arising from frequent plasma exchange have been known for a long time from blood transfusions as allergic reactions up to anaphylactoid reactions. A special manifestation was described as the "transfusion lung".
- 20 c) the risk of viral infection is present (HIV, hepatitis).
- d) in FHF the organism answers the massive cell degradation with an increased secretion of growth hormones in order to enhance organ regeneration. These growth hormon levels are
25 higher than in healthy persons and are therefore not present in the donor plasma.
- e) plasma exchange is expensive.

30 7. Exchange transfusions

In severe newborn-hyperbilirubinemia today exchange transfusions are the treatment methods of choice. The therapy is helpful to the patient because irreversible brain

damage is prevented. However, the transfusions have similar disadvantages as mentioned above for plasma exchange.

8. Other extracorporeal methods

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Different other approaches especially towards the field of FHF treatment and with respect to the removal of protein-bound toxins have been made in the past years:

- 10 a) charcoal-impregnated hollow fibers/dialysis against sorbent suspensions

Trials to combine the hemocompatibility of dialysis with the effectivity of charcoal adsorption by filling charcoal into cellulosic membranes or recirculate adsorbent suspensions

- 15 (powdered charcoal and ion exchange resins) in order to remove "middle molecules" did not at all or not sufficiently come up to the expectations. Especially the removal of strongly protein-bound fractions of ABT (e.g. unconjugated bilirubin) was insufficient. The reasons for this, in our
20 opinion, was the use of membranes which lack the predispositions for the "tunneling effect" which will be described below.

b) lipophilic liquid membranes

- 25 A further effort was made to remove protein-bound toxins by the use of hydrophobic semipermeable polymer membranes filled with lipophilic liquids enabling the passage of water-insoluble compounds like middle-molecular weight fatty acids and mercaptans. An alkaline dialysate (pH 13) solution
30 was proposed as acceptor solution for these toxins. The disadvantages of this method are the following:

1. Some of the ABTs are insoluble in this liquid membrane and are therefore excluded from the removal process (e.g. unconjugated bilirubin).

5 2. The insolubility of hydrophilic toxins (e.g. ammonia) in the liquid phase excluded their removal, too, and required the additional use of a further dialysis procedure, because most of the above described intoxications were of complex nature including hydrophilic and hydrophobic toxins. By the
10 use of an additional dialysis device the extracorporeal blood volume as well as blood-polymer contact increases. Last but not least costs are very high.

3. The use of alkaline dialysate solutions might be of
15 potential harm to the patient influencing the endogenous pH value at least in cases of membrane damage.

9. Biohybrid systems

20 These systems are bioreactors designed for the processing of blood or plasma which include biological components (e.g. liver slices, liver cells) which will provide a natural detoxification. Because of the limited functionality and short life of liver slices only cell-based systems are
25 acceptable. The disadvantages of this method are:

a) There is no stable and save source providing sufficient amounts of cells needed for the treatment.

The most important cell for the detoxification of protein-bound toxins, the hepatocyte of the liver until today could
30 not be produced by cultivation in vitro without genetic manipulation which is followed by cell alteration and therefore connected with further risks that cannot be calculated. The use of tumor liver cells which could be

produced by in vitro cultivation is connected with the risk of developing tumor and furthermore with risks arising from a metabolic alteration of the cells which cannot be foreseen. The use of animal cells is connected with immunological reactions between the plasma/blood of the patient on the one hand and the xenogenic liver cell on the other.

b) Difficulty of the method.

Because of the poor possibilities for cryoconservation of the cells or complete bioreactors the preparation of the device has to be done always just prior to use. This makes this method usable only for highly specialized centers.

c) High costs.

All steps of this treatment involve high costs.

10. Liver transplantation

Today this is the method of choice in patients with either chronic or acute end stage liver failure because it shows the best long time survival rates from all therapies mentioned for liver failure. The limiting factors of this method are limited availability of suitable donor organs (long waiting lists), surgery risk, life-long immune suppressive therapy to avoid rejection associated with the common complications (e.g. high infection risk, high tumor risk, risk of diabetes). Moreover, the indications for transplantation are very tight, automatically excluding a large number of fatally ill patients. Thus, transplantation did not avoid the need for sufficient detoxification procedures for those patients not eligible for surgery. A number of those patients eligible for surgery is in a status too bad to undergo surgery because of FHF with coma,

hypotension, high bleeding risk. These patients are in need for a sufficient "liver bridging treatment" to prepare for transplantation and, in a number of cases, when patients come into FHF after transplantation it is needed again. Last
5 but not least transplantation causes high costs arising from surgery as well as from the immune suppressive therapy.

Therefore, it would be useful to provide a method for the separation of protein-bound substances such as ABTs from
10 protein-containing liquids, e.g. from blood or plasma of patients suffering e.g. from FHF, chronic liver insufficiency, accidental or suicidal drug overdosage and ESRD, which is effective, safe and less expensive. It would be advantageous if this method did not interfere with the
15 homeostasis of the liquid, e.g. blood, for instance, by undesired removal of valuable components, e.g. proteins, from the liquid or addition of potentially harmful (toxic) substances to the liquid.

20 Thus, one object of the present invention is to provide a method for the separation of protein-bound substances from a protein-containing liquid containing these substances by dialysis. Another object of the invention is to provide a membrane for the separation of said protein-bound substances
25 from a protein-containing liquid containing these substances by dialysis.

Further objects will become apparent from the following description, drawings and claims.

Summary of the invention

- The invention is based on the unexpected finding that protein-bound substances (PBS), including even those being
5 strongly bound, can be removed from protein-containing liquids (A) by dialysis against a dialysate liquid (B) and by means of a semipermeable membrane and by means of a protein having an acceptor function for the PBS.
- 10 Preferably, the dialysate liquid (B) of the present invention contains a protein having an acceptor function for the protein-bound substance (PBS) to be removed from the protein-containing liquid (A), in general of the type of protein present in the PBS-protein complex in liquid (A).
- 15 In case of plasma or blood as protein-containing liquid (A) the preferred acceptor protein in the dialysate liquid (B) is albumin, in particular human serum albumin or recombinant human albumin.
- 20 The membrane of the present invention preferably comprises two functionally different parts. One part has the actual separating membrane function permitting the PBS and, if present, the water-soluble substances to pass through under the conditions of the process of the present invention and
25 excluding the protein(s) which had bound the PBS in liquid (A) and the acceptor protein of liquid (B), and the other part has a port- and adsorption function. Preferably, the membrane is coated with a protein having an acceptor function for the PBS. In a preferred embodiment the membrane
30 of the present invention comprises a tunnel-like structure facing the liquid (A) side, the tunnels having a length less than about 10 μm and having a diameter sufficiently small to exclude the protein in liquid (A), and a port- and adsorption-structure on the dialysate liquid (B) side.
- 35 Preferably, the membrane is coated on at least one side,

preferably the dialysate liquid (B) side, with a thin film of a protein having an acceptor function for the protein-bound substances.

- 5 The membrane of the present invention may have the macroscopic form of a flat film, a thin-walled but large diameter tube, or preferably fine hollow fibers. Membrane technology, hollow-fiber membranes, and dialysis is described in Kirk-Othmer, Encyclopedia of Chemical
10 Technology, third edition, Vol. 7 (1979), 564-579, in particular 574-577, Vol. 12 (1980), 492-517 and Vol. 15 (1981), 92-131. Furthermore, membranes and membrane separation processes are described in Ullmann's Encyclopedia of Industrial Chemistry, Fifth edition, Vol A 16 (1990),
15 187-263.

The matrix material for the membrane may be made from many materials, including ceramics, graphite, metals, metal oxides, and polymers, as long as they have an affinity
20 towards the protein on the liquid (A) and the dialysate liquid (B) side. The methods used most widely today are sintering of powders, stretching of films, irradiation and etching of films and phase inversion techniques. The preferred materials for the membranes of the present
25 invention are organic polymers selected from the group consisting of polysulfones, polyamides, polycarbonates, polyesters, acrylonitrile polymers, vinyl alcohol polymers, acrylate polymers, methacrylate polymers, and cellulose acetate polymers. Especially preferred are polysulfone
30 membranes hydrophilized with e.g. polyvinylpyrrolidone.

A precise and complete definition of a membrane is rather difficult; see Ullmann, loc. cit., page 190-191, No. 2.1 and 2.2. A membrane can be homogeneous, microporous, or
35 heterogeneous, symmetric or asymmetric in structure. It may

be neutral, or may have functional groups with specific binding or complexing abilities. The most important membranes currently employed in separation processes are the asymmetric membranes; see Ullmann, loc. cit., page 219 et seq., No. 4.2. Known asymmetric membranes have a "finger"-type structure, a sponge-type structure with a graded pore size distribution or a sponge type structure with a uniform pore size distribution; see Ullmann, loc. cit., page 223-224.

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The most preferred membrane structure of the present invention is an asymmetric membrane composed of a thin selective skin layer of a highly porous substructure, with pores penetrating the membrane more or less perpendicularly in the form of fingers or channels from the skin downward. The very thin skin represents the actual membrane and may contain pores. The porous substructure serves as a support for the skin layer and permits the protein having an acceptor function to come close to the skin and to accept the protein-bound substances penetrating the skin from the liquid (A) side towards the dialysate liquid (B) side.

Prior to the separation procedure the membrane is preferably prepared as follows. The membrane is treated from the liquid (A) side and/or from the liquid (B) side with a liquid which contains the protein having an acceptor function, preferably a 0.9% NaCl solution, containing the acceptor protein, preferably human serum albumin in a concentration from about 1 to about 50 g/100 ml, more preferably from about 5 to about 20 g/100 ml. The treatment time is about 1 to about 30 min, preferably about 10 to about 20 min, at a temperature from about 15 to about 40 °C, preferably from about 18 to about 37 °C.

The method of the present invention for the separation of protein-bound substances and, of course conventional water-soluble substances that may be present, from a protein containing liquid (A) is carried out as follows:

5

The liquid (A) to be purified is passed through a dialyzer comprising a membrane along the liquid (A) side of the membrane with a flow rate of about 50 to about 500 ml/min, preferably about 100 to about 200 ml/min per one sqm
10 membrane area on the liquid (A) side. The dialysate liquid (B) is passed along the dialysate liquid (B) side of the membrane with a flow rate of about 50 to about 500 ml/min, preferably of about 100 to about 200 ml/min per one sqm membrane area and preferably with the same flow rate as the
15 liquid (A).

The dialysate liquid (B) obtained and containing the protein-bound substances and possibly water-soluble substances from liquid (A) preferably is then passed through a second conventional dialyzer that is connected to a
20 conventional dialysis machine. A dialysis against an aqueous standard dialysate is carried out. By this dialysis water-soluble substances are exchanged between the dialysate liquid (B) and the standard dialysate. Thus, water-soluble toxins such as urea or creatinine can be separated from the
25 dialysate liquid (B) and electrolytes, glucose and pH can be balanced in the dialysate liquid (B) and, therefore, also in liquid (A). The dialysate liquid (B) obtained free from water-soluble substances preferably is then passed through a charcoal-adsorbent, e.g. Adsorba 300 C from GAMBRO AB or
30 N350 from ASAHI, and an anion exchange column, e.g. BR350 from ASAHI, to remove the protein-bound substances from the protein acceptor in the dialysate liquid (B). The purified dialysate liquid (B) obtained is then returned to the

dialysate liquid (B) side of the membrane of the present invention and reused.

Other advantages and benefits will be apparent to those skilled in the art from the detailed description that follows.

Description of the drawings

Fig. 1 is a graph showing the results of an in vitro separation of protein-bound substances (unconjugated bilirubin, free fatty acids, phenol, sulfobromophthalein) from plasma (liquid (A)) in accordance with the method of the present invention. The decrease of the protein-bound substances (PBS) is shown in percent of the initial concentrations in the plasma (liquid (A)).

Fig. 2 is a graph showing the increase of protein-bound substances in dialysate solution (liquid (B); corresponding to Fig. 1 and obtained from the same experiment) in percent of the initial concentrations in the plasma (liquid (A)).

Fig. 3 is a graph showing total serum bilirubin concentrations in a 29 year old patient with an acute exacerbation of a chronic liver insufficiency during a 4 days period of MARS treatment followed by a 2 days conventional HDF.

Fig. 4, 5 and 6 illustrate embodiments for the purification of blood by means of hemodialysis according to the present invention.

DA - Dialyzer A (albumin coated Membrane)
DB - Dialyzer B (conventional dialyzer)
ADS A - Adsorbent A

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ADS B - Adsorbent B

Pump 1 - Blood roller pump

Pump 2 - first albumin dialysate roller pump

Pump 3 - second albumin dialysate roller pump

5 I - V - Safety devices:

I - Blood line bubble snare

II - Albumin dialysate line bubble snare

III - Blood lack detector

IV - Veneous drop chamber

10 V - Safety clamp

in/out - Blood inflow and outflow

X/Y - Albumin dialysate flow connectors (DA)

U/V - Conventional dialysate flow connectors (DB)

The blood (liquid (A)) flows from "in" pumped by pump
15 through the bubble snare into the dialyzer (DA) and after
passing it through the veneous drop chamber, after that
passing the safety clamp. Safety devices are not part of the
invention but have to be used because of safety regulations.
The albumin dialysate (liquid (B)) is pumped by roller pump
20 2 from connector X in direction to connector Y or from Y to
X, but preferably counter-currently to the blood flow,
passing at first the blood lack detector (III) and also a
bubble snare (II) . Thereafter, liquid (B) passes a
conventional dialyzer (DB) and, if desired, adsorbent
25 columns (A and B).

Other possibilities are:

- The liquid (B) passes only through adsorbents and not
through a dialyzer

30 - The liquid (B) passes only through dialyzer DB and not
through adsorbents.

The conventional dialyzer (DB) is connected to a
conventional dialysate liquid which may be coming from a
conventional dialysis machine or may be pumped by other
perfusor or roller pumps from bags.

Detailed description of the invention

A. Method for separating protein-bound substances from protein-containing liquids

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The present invention provides a practical and effective method for the removal of undesired or potentially harmful protein-bound substances and/or lipophilic substances from protein-containing liquids such as plasma and blood.

10 The basic procedure is similiar to conventional high flux dialysis with modifications according to the present invention as described below.

1. The dialysate liquid (B)

15

The dialysate liquid (B) contains a protein serving as an acceptor for the protein-bound substances (PBS) to be removed from the liquid (A). The acceptor protein should have a sufficient affinity towards the substances which are bound to the protein in liquid (A). A preferred acceptor protein is human serum albumin. The concentration of the acceptor protein is from about 1 to about 50 g/100 ml, preferably from about 6 to about 40 g/100 ml, more preferably from about 8 to about 30 g/100 ml and most preferably from about 8 to about 20 g/100 ml.

The dialysate liquid (B) contains furthermore salts like NaCl, KCl, MgCl₂, CaCl₂, sodium lactate and glucose monohydrate in amounts depending on the electrolyte composition in the blood of the specific patient. For example, in the dialysis of a patient suffering hypopotassemia a higher concentration of potassium ions is required.

Preferred ion concentrations in a dialysate liquid (B) that is bicarbonate buffered are for sodium from about 130 to about 145 mmol/1000 ml, for calcium from about 1.0 to about 2.5 mmol/1000 ml, for potassium from about 2.0 to about 4.0 mmol/1000 ml, for magnesium from about 0.2 to about 0.8 mmol/1000 ml, for chloride from about 100 to about 110 mmol/1000 ml, for bicarbonate from about 30 to about 40 mmol/1000 ml, for acetate from about 2 to about 10 mmol/1000 ml, for human serum albumin from about 1 to about 50 g/100 ml, preferably from about 6 to about 40 g/100 ml, more preferably from about 8 to about 30 g/100 ml, and most preferably from about 8 to about 20 g/100 ml.

More preferred ion concentrations in a dialysate liquid (B) that is bicarbonate buffered are for sodium from about 135 to about 140 mmol/1000 ml, for calcium from about 1.5 to about 2.0 mmol/1000 ml, for potassium from about 3.0 to about 3.5 mmol/1000 ml, for magnesium from about 0.4 to about 0.6 mmol/1000 ml, for chloride from about 104 to about 108 mmol/1000 ml, for bicarbonate from about 34 to about 38 mmol/1000 ml, for acetate from about 4 to about 8 mmol/1000 ml, for human serum albumin from about 1 to about 50 g/100 ml, preferably from about 6 to about 40 g/100 ml, more preferably from about 8 to about 30 g/100 ml, and most preferably from about 8 to about 20 g/100 ml.

Preferred ion concentrations in a dialysate liquid (B) that is acetate buffered are for sodium from about 130 to about 145 mmol/1000 ml, for calcium from about 1.0 to about 2.5 mmol/1000 ml, for potassium from about 2.0 to about 4.0 mmol/1000 ml, for magnesium from about 0.2 to about 0.8 mmol/1000 ml, for chloride from about 100 to about 110 mmol/1000 ml, for acetate from about 30 to about 40 mmol/1000 ml, for human serum albumin from about 1 to about 50 g/100 ml, preferably from about 6 to about 40 g/100 ml,

more preferably from about 8 to about 30 g/100 ml, and most preferably from about 8 to about 20 g/100 ml.

More preferred ion concentrations in a dialysate liquid (B) that is acetate buffered are for sodium from about 135 to about 140 mmol/1000 ml, for calcium from about 1.5 to about 2.0 mmol/1000 ml, for potassium from about 3.0 to about 3.5 mmol/1000 ml, for magnesium from about 0.4 to about 0.6 mmol/1000 ml, for chloride from about 104 to about 108 mmol/1000 ml, for acetate from about 33 to about 38 mmol/1000 ml, for human serum albumin from about 1 to about 50 g/100 ml, preferably from about 6 to about 40 g/100 ml, more preferably from about 8 to about 30 g/100 ml, and most preferably from about 8 to about 20 g/100 ml.

An example for a dialysate liquid (B) comprises from about 10 to about 20% by weight human serum albumin, about 6.1 g NaCl, about 4.0 g sodium lactate, about 0.15 g KCl, about 0.31 g $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$, 0.15 g $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$, and 1.65 g glucose monohydrate per liter of dialysate liquid (B).

2. The membrane

The membrane of the present invention preferably comprises two functionally different parts (regions). One part has the actual separating membrane function permitting the PBS and the water-soluble substances to pass through under the conditions of the process of the present invention and excluding the protein(s) which had bound the PBS in liquid (A) and the acceptor protein of liquid (B), and the other part has a port- and adsorption function. Preferably, the membrane is coated with a protein having an acceptor function for the PBS. In a preferred embodiment the membrane of the present invention comprises a thin layer of a tunnel

like structure facing the liquid (A) side, the tunnels having a length less than about 10 μm , preferably less than about 5 μm , more preferably less than about 0.1 μm and most preferably between about 0.01 and about 0.1 μm . The tunnels
5 have a diameter sufficiently small to exclude the protein in liquid (A), preferably to permit the passage of molecules having a molecular weight from about 20,000 daltons to about 66,000 daltons, more preferably from about 50,000 to about 66,000 daltons through the tunnels. Preferably the sieve
10 coefficient of the membrane with respect to the protein in liquid (A) is less than 0.1, more preferably less than 0.01. Furthermore, the membrane preferably comprises a port- and adsorption-structure on the dialysate liquid (B) side. This part has to provide a structure sufficiently open to permit
15 the acceptor protein in the dialysate liquid (B) to enter the port- and adsorption layer to accept the PBS coming from the liquid (A) side of the membrane. Moreover the internal surface of this part acts as an adsorber for the PBS via the acceptor protein that is adsorbed by the coating procedure
20 described in the following or by other structures suitable for binding the PBS. This adsorption can either be stable over time or reversible. Preferably the membrane is coated on at least one side with a thin film of a protein having an acceptor function for the protein-bound substances. A
25 commercial dialyzer comprising a membrane of the present invention may contain on the liquid (B) side a solution of the acceptor protein.

The membrane of the present invention may have the
30 macroscopic form of a flat film, a thin-walled but large diameter tube, or preferably fine hollow fibers.

The matrix material for the membrane may be made from various materials, including ceramics, graphite, metals,
35 metal oxides, and polymers, as long as they have an affinity

towards the protein on the liquid (A) and the dialysate liquid (B) side. The methods used most widely today are sintering of powders, stretching of films, irradiation and etching of films and phase inversion techniques. The preferred materials for the membranes of the present invention are organic polymers selected from the group consisting of polysulfones, polyamides, polycarbonates, polyesters, acrylonitrile polymers, vinyl alcohol polymers, acrylate polymers, methacrylate polymers, and cellulose acetate polymers.

The preferred polymer membranes used in the present invention are highly permeable asymmetric polysulfone membranes hydrophilized with e.g. polyvinylpyrrolidone, e.g. HF 80 of Fresenius AG.

Such membranes and membrane modules, dialysis cardruges, artificial kidney membrane systems are commercially available for instance from Fresenius AG, Polyflux from GAMBRO AB, CT190G from Baxter Inc.

First part: The layer or structure of the membrane facing the liquid (A) side has to provide the actual membrane permitting a selective transfer of protein-bound substances and water-soluble substances, i.e. low-molecular substances and "middle molecules" from the liquid (A) side to the dialyzing solution (liquid (B) side). Thus, an effective net transport of undesired substances occurs from the liquid (A) side to the dialysate liquid (B) side following the concentration gradient for the undesired substances decreasing from the liquid (A) side towards the dialysate liquid (B) side. Three conditions have to be met for the actual membrane:

1. The tunnels have to be sufficiently short, preferably less than about 5 μm , more preferably less than about 1 μm , and most preferably less than about 0,1 μm .
 2. The tunnel diameter has to be sufficiently large to permit passage of the undesired molecules and sufficiently small to inhibit passage of the desired molecules contained in liquid (A) towards liquid (B) and of the acceptor protein from liquid (B) to liquid (A). In case of plasma or blood as liquid (A) the exclusion limit is preferably about 66,000 daltons. Preferably the sieve coefficient of the membrane with respect to the protein in liquid (A) is less than 0.1, more preferably less than 0.01.
 3. The chemical, physical etc. structure of the layer or structure of the actual membrane facing the liquid (A) side is such that passage of the undesired substances is permitted, e.g. by hydrophobic and hydrophilic microdomains.
- Second part: The layer or structure of the membrane facing the liquid (B) side has to provide a more open membrane structure normally in a sponge- or finger-like fashion. This part provides an important port- and adsorption-function within this part of the membrane:
1. Due to the open-spaced structure of this part of the membrane the acceptor protein coming from the dialysate liquid (B) side can approach the dialysate side ostium of the structure facing the liquid (A) side described above and accept undesired substances, such as protein-bound substances passing through the tunnel-like structure from the liquid (A) side.
 2. Due to the large total surface area present in this structure it adsorbs remarkable amounts of the protein-bound

substances (PBS) via attached molecules that function as a kind of spacer in this mediate membrane adsorption or the PBS are directly membrane bound if the membrane has a capacity to adsorb the PBS due to its own structure. This
5 adsorption can either be reversible or irreversible but preferably it is reversible.

3. Due to the open structure towards the dialysate liquid (B) side of the membrane a dialysate movement that might be
10 directed perpendicular or in parallel to the outer membrane surface or in a different fashion can transport acceptor protein molecules both into the port layer and out of the port layer. Preferably the movement and the transport perpendicular to the outer membrane surface is provided by
15 an alternating influx and outflux movement of liquid (B) that moves into the port membrane and back out into the liquid (B) stream. This influx and outflux can be provided by a pulse-like pressure profile obtained by the use of roller pumps or a change in transmembranal pressure changing
20 along the membrane from being directed towards the liquid (B) first (positive TMP) and to the liquid (A) at last (negative TMP); TMP = transmembranal pressure.

Thus, the dialysis membrane of the present invention
25 preferably is functionally divided into a tunnel-like part and a finger- or sponge-like port/adsorption part. Both of them have to fulfill certain prerequisites to render the method of the present invention possible. The ideal tunnel-like part would be one with a length next to zero (0.01 to
30 0.1 μm), a diameter next to the size of the desired protein to be purified and kept in the retentate, e.g. the diameter of albumin. In other words, the tunnel-like part should have a diameter sufficiently small to retain valuable and desired substances of the liquid (A) in the retentate and to permit
35 protein-bound substances and other undesired substances

contained in liquid (A) to pass to the dialysate liquid (B) side.

5 The ideal port/adsorption part of the dialysis membrane of the present invention has a very open structure to enable the acceptor protein to approach and leave the area next to the dialysate side of the tunnel. It has a large inner surface which adsorbs the PBS directly or via the attached acceptor protein. The total diameter of this part should
10 again be as small as possible to render the exchange into the dialysate stream more effective. The latter two points can be brought to their extremes almost excluding the other one according to whether more adsorption or more transit through the port/adsorption part of the membrane is desired.

15 Conventional dialysis membranes for purifying e.g. plasma or blood can be classified by functional or structural criteria. Functional criteria are high flux, low flux or highly permeable, whereas structural criteria are e.g. flat,
20 hollow fiber, symmetric or asymmetric. The group of tunnel-like membranes (TM) useful for the present invention is not sufficiently described by these terms because

- 25 a) TM are high flux and highly permeable membranes but not every high flux membrane named "highly permeable" is a TM (e.g. AN69 from HOSPAL);
- b) TM can be asymmetric but not every asymmetric membrane is a TM (e.g. F8 from FRESSENIUS AG);
- c) TM can be asymmetric and highly permeable but not every
30 asymmetric and highly permeable membrane is a TM (PMMA from Toray)
- d) TM can be symmetric but not every symmetric membrane is a TM (e.g. Cuprophane from AKZO).

Therefore the term tunnel-like membrane represents a new quality of structural and functional features of dialysis membranes useful for the present invention.

5 4. Pretreatment and conditions of the membrane

The membrane of the present invention preferably is pretreated as follows. The membrane is impregnated on at least one side, preferably both from the liquid (A) side and
10 from the liquid (B) side with a solution of the acceptor protein. A preferred solution for the impregnating step is a 0.9% NaCl solution, containing the acceptor protein, preferably human serum albumin, in a concentration from about 1 to about 50 g/100 ml, preferably from about 6 to
15 about 40 g/100 ml, more preferably from about 8 to about 30 g/100 ml, and most preferably from about 8 to about 20 g/100ml. The impregnating solution is passed along the liquid (A) side and the liquid (B) side of the membrane for a time sufficient to permit penetration and adsorption of
20 the acceptor protein on the two parts of the membrane, in general from about 1 to about 120 min, preferably from about 10 to about 60 min, at a temperature from about 15 to about 40°C, preferably from about 18 to about 37°C, the pH value being from about 5 to about 9, preferably about 7. The
25 pretreatment can be carried out immediately prior to use of the membrane, but the pretreated membrane may also be stored under sterile conditions at a temperature up to 24°C for up to two years if the acceptor protein is human serum albumin.

30 Preferably the impregnating solution is pumped by roller pumps exhibiting a "pulse like pressure profile" during the coating procedure, e.g. by two roller pumps, one on the dialysate side compartment and one on the blood side compartment of the dialyzer. Preferably there is a phase

delay between the pressure profiles of the two pumps thus to ensure an effective in- and outflow of the solution on both sides of the membrane.

5 5. Procedure for the separation of protein-bound substances (PBS) from a protein containing liquid (A)

The procedure for the separation of PBS from a protein containing liquid (A) preferably is carried out as follows:

10

Liquid (A) to be purified is passed along the liquid (A) side of the dialysis membrane of the present invention with a flow rate from about 50 to about 300 ml/min, preferably from about 100 to about 200 ml/min per sqm of the dialysis
15 membrane. The dialysate liquid (B) is passed along the dialysate side (B) of the membrane with a flow rate from about 50 to about 1000 ml/min, preferably from about 100 to about 500 ml/min per sqm of the dialysis membrane. The flow rates of the liquid (A) and thus liquid (B) are preferably
20 in the same order of magnitude. The ratio of the flow rate of liquid (A) to liquid (B) is from about 1 : 0.1 to about 1 : 10, preferably from about 1 : 1 to about 1 : 5. The retentate is the purified protein-containing liquid (A) from which protein-bound substances and other undesired
25 substances are removed.

In a preferred embodiment of the process of the present invention the first dialysis step of the liquid (A) is combined with two steps of aftertreatment of the dialysate
30 liquid (B) obtained.

First the dialysate liquid (B) obtained is passed through a second conventional dialyzer which is connected to a conventional dialysis machine. Dialysis is carried out

against an aqueous standard dialysate liquid. By this dialysis water-soluble substances can be exchanged between the dialysate liquid (B) and a standard dialysate liquid. Water-soluble toxins, urea and/or creatinine are removed
5 from the dialysate liquid (B), and electrolytes, glucose and the pH value can be balanced in the dialysate liquid (B) which is the retentate. The dialysate liquid (B) is thereafter passed through a charcoal-adsorbent, e.g. Adsorba 300 C from GAMBRO AB or N350 from ASAHI, and then through an
10 anion exchange column, e.g. BR350 from ASAHI, to remove the protein-bound substances from the acceptor protein in the dialysate liquid (B). The purified acceptor protein-containing dialysate liquid (B) is then returned to the liquid (B) side of the membrane of the present invention.

15 This procedure has been tested experimentally for the separation of albumin-bound drugs and toxins in a protein-containing liquid and led to a significant reduction of these compounds in the liquid.

20 Other possible simplified embodiments of the procedure of the present invention comprise the following modifications. The dialysate liquid (B) coming from the dialyzer may be passed through another dialyzer but not through any
25 adsorbent. The dialysate liquid (B) coming from the dialyzer may be passed through one or two adsorbents but not through another dialyzer. The dialysate liquid (B) coming from the dialyzer may be pumped directly back into the inlet of the dialysate compartment of the dialyzer (e.g. by a roller
30 pump) thus realizing a sufficient movement of the dialysate liquid (B) and sufficient removal of ABT. A further simple modification would be a dialyzer with a dialysate compartment filled with the dialysate liquid (B) comprising human serum albumin in a concentration of from about 1 to about 50 g/dl,
35 preferably from about 6 to about 40 g/dl, more preferably

between 8 and 30 g/dl, and most preferably from about 8 to about 20 g/dl that is closed at the dialysate inlet and outlet. The whole dialyzer may be moved, e.g. by shaking or rolling.

5 The benefit of the method of the present invention is that a biological protein-containing liquid such as blood or plasma contaminated with potentially harmful or undesired proteinbound substances and possibly undesired water-soluble
10 substances can be selectively purified by the method of this invention so that the biological liquid contains the protein-bound substance and the other undesired substances in a lower concentration than before and does not exhibit the potentially harmful or unwanted effects which it had
15 prior to the dialysis treatment.

Another benefit of the method of the present invention is that the chemical and physical homeostasis of the biological liquid remains almost unchanged, i.e. the method and the
20 dialysis membrane used for it exert good biocompatibility.

The method of the present invention furthermore has the advantage that it is simple, practical and suitable for treating large volumes of biological liquids, e.g. blood in
25 an extracorporeal circuit repeatedly for hours and for different conditions with commercially available dialysis machines.

A disposable set for the separation of protein-bound
30 substances and water-soluble substances from plasma or blood containing said substances can comprise a membrane as described before, a second conventional dialyzer for hemodialysis, a conventional charcoal adsorber unit for hemoperfusion, and a conventional ion exchange resin unit

for hemoperfusion interconnected by tubing and a unit of a human serum albumin containing dialysate liquid.

5 A disposable set for the separation of protein-bound substances and water-soluble substances from plasma or blood containing said substances may as well comprise a dialyzer having a membrane as described before and being filled on the dialysate liquid (B) side with a human serum albumin containing liquid, a second conventional dialyzer for 10 hemodialysis, a conventional charcoal adsorber unit for hemoperfusion, and a conventional ion exchange resin unit for hemoperfusion interconnected by tubing and a unit of a human serum albumin containing dialysate liquid.

15 B. Evaluation of removal of PBS and water soluble substances

All compounds were determined immediately after the experiment in order to avoid degradation especially of the light instable bilirubin.

20

- Bromosulfophthalein: Spectrophotometrically according to the method of BECH

- Bilirubin: a) Spectrophotometrically according to the method of JENDRASSIK; b) KODAK EKTACHEM dry chemistry.

25

Both methods showed good correlation in the range of concentrations used.

- Phenols: a) Spectrophotometrically according to the method of BECHER; b) HPLC. Again both methods showed good correlation in the range of concentrations used.

30

- Free fatty acids according to the method of LAURELL and TIBBLING (i.e. fatty acids that have not formed esters)

- Digoxin: ABBOTT analyzer/dry chemistry

- Digitoxin: ABBOTT analyzer/dry chemistry

- creatinine, urea, uric acid, transaminases, blood cell counts: KODAK EKTACHEM dry chemistry.

C. The in vitro model

5

A conventional dialysis machine with two closed loop compartments (a plasma compartment and a compartment for dialysis solution) is used in order to permit the measurement not only of the decreasing concentrations in the biological liquid but also the increasing concentrations in the dialysate which is the essential criterion to distinguish a membrane transport process from an adsorption to the membrane. Two glass bottles about 25 cm high and about 10 cm diameter with a volume of 500 ml each are used, one as container for the biological liquid containing plasma the other as container for the dialysate solution. Both bottles have connectors for two tubes, one for the inflow and one for the outflow of plasma or dialysate, respectively. The distance between the inflow and the outflow should not be smaller than 10 cm in order to avoid "shunt circulation". The two connectors of each bottle are connected with two flexible plastic tubes (about 100 cm and about 6 mm diameter) each with a silicone segment permitting the use of a conventional roller pump for dialysis. The other end of the two tubes from the bottle containing the plasma are connected with the connectors of the blood side of a dialysator in the following manner: the outflow of the bottle is connected with the inflow of the dialysator, the outflow of the dialysator is connected with the inflow of the bottle. The other end of the two tubes from the bottle containing the dialysate are connected with the connectors of the dialysate side of the dialysator in the following manner: The outflow of the bottle is connected with the dialysate inflow of the dialysator and the dialysate outflow

of the dialysator is connected with the inflow of the bottle. The plasma inflow of the dialysator is placed next to the dialysate outflow in order to design the dialysate flow through the dialysator countercurrently to the plasma flow as it is known from clinical hemodialysis. The silicone segments of the inflow/outflow tubes of the plasma bottle are used to pump the plasma by a simultane roller pump. Also, the silicone segments of the inflow/outflow tubes of the dialysate bottle are used to pump the dialysate by a simultane roller pump. In this way the inflow/outflow volumes of both compartments are balanced and transmembranal water losses which could influence the results are avoided.

Preparation of the plasma used for in vitro experiments

a) For evaluation of the method of the present invention using a polysulfone dialysis membrane

Human heparinized plasma was taken from young male donors and enriched with model substances with a high protein binding rate:

- caprylic acid (750 mg/1000 ml)
- phenol (530 mg/1000 ml)
- unconjugated bilirubin (11 mg/100 ml)
- sulfobromophthalein (230 mg/1000 ml)

110 mg unconjugated bilirubin, 230 mg sulfobromophthalein, 750 mg caprylic acid, and 530 mg phenol were dissolved in 50 ml of a 0.1 M NaOH solution. Thereafter 2.5 g human albumin (fatty acid free, SIGMA) were dissolved in the solution. Thereafter the pH value was adjusted to 7.4 by addition of a 30 percent acetic acid solution. Thereafter this toxin cocktail was mixed with 950 ml heparinized plasma. 500 ml of this plasma solution were filled into the plasma bottle.

Composition of the dialysate: 100 ml of a 20 percent (20 g/100 ml) human albumin solution were mixed with 400 ml of a commercial CVVH dialysis solution. 500 ml of this albumin containing solution were filled into the dialysate bottle.

5

b) For evaluation of other dialysis membranes/hemofilters

Human heparinized plasma was taken from young male donors and enriched with model substances with a high protein-binding rate:

10

-unconjugated bilirubin (11 mg/100 ml)

-sulfobromophthaleine (230 mg/1000 ml)

as well as with model substances which are known as markers of uremia and effectivity of dialysis:

15

-creatinine (6 mg/100 ml)

-urea (100 mg/100 ml)

20 -uric acid (17 mg/100 ml)

110 mg unconjugated bilirubin, 230 mg sulfobromophthalein, 60 mg creatinine, 1000 mg urea, and 170 mg uric acid were dissolved in 50 ml of a 0.1 M NaOH solution. Thereafter 2.5 g human albumin (fatty acid free, SIGMA) were dissolved in the solution. Thereafter the pH value was adjusted to 7.4 by addition of a 30 percent acetic acid solution at room temperature. Thereafter this toxin cocktail was mixed with 950 ml heparinized human plasma. 500 ml of this plasma solution were filled into the plasma bottle.

25

30

After filling the bottles they were closed hermetically and the pump segments (inflow and outflow) installed to the simultaneous roller pumps in a manner permitting recirculation of each (plasma and dialysate) circle counter-currently. The

35

flow rates were adjusted at 100 ml/min. The temperature was adjusted to 37 °C.

The following dialysis membranes/hemofilters were used:

5

a) For the evaluation of the principle of the method of the present invention

HF 80, FRESenius AG, Germany

10

b) For the evaluation of other dialysis membranes

Diafilter 30, AMICON, U.S.A.

FH 88, GAMBRO AB, Sweden

15 LUNDIA PRO 5, GAMBRO AB, Sweden

CT 110 G, BAXTER, U.S.A.

SPAN, ORGANON TECHNIKA, Netherlands

KF 201 EVAL N 13, KAWASUMI, Japan

Bl-2.1U, TORAY Medical Co., Japan

20 FILTRAL 16, HOSPAL, France

Dialysis membranes, technical information from the firms:

25 *The highly permeable FRESenius AG polysulfone membrane is a asymmetric membrane with a 0.5 to 1 um skin layer determining the cut off (about 40,000 daltons). The skin layer is supported by a macroporous support layer of about 40 um thickness for the High flux dialysis membrane and of about 35 um thickness for the hemofiltration membrane. The
30 hydrophobic surface structure of the polysulfone membrane is hydrophilized by polyvinylpyrrolidone.

The low permeable FRESenius AG polysulfone hollow fiber membrane is an asymmetric membrane with a 0.5 to 1 um skin layer determining the cut off (ca 5000 daltons) which is

supported by a macroporous support layer of about 40 um thickness. The hydrophobic surface structure of the polysulfone membrane is hydrophilized by polyvinylpyrrolidone. These fibers are present in the following Fresenius dialyzers: F3, F4, F5, F6, F7, F8.

*The HOSPAL PAN membrane (AN 69) is a symmetric membrane based on polyacrylonitrile with a high permeability and a membrane thickness of 50 um. This membrane is present in Filtral 8-20 as a hollow fiber and in Biospal.

*The ORGANON TECHNIKA SPAN membrane is an asymmetric membrane based on polyacrylonitrile.

*The DIAFILTER is an asymmetric hollow fiber polysulfone hemofilter membrane with a skin layer determining the cut off supported by a macroporous support layer. This hemofiltration membrane is present in the following AMICON filters: Minifilter, Minifilter plus, Diafilter 10, 20, 30, and 50.

*The Lundia pro 5 membrane is a flat polycarbonate dialysis membrane.

*The GAMBRO AB polyamide hollow fiber membranes are symmetric membranes sold in three modifications:

- Polyamide for high flux dialysis and hemodiafiltration with a wall thickness of 50 um, designed as a 3-layer and of high hydrophobicity. The internal diameter of the hollow fiber is 220 um. These hollow fibers are present in Polyflux 130 and Polyflux 150 (Trademark names).
- Polyamide for hemofiltration with a wall thickness of 60 um, designed as a 3-layer and of a high hydrophilicity. The internal diameter of the hollow fiber is 215 um. These

hollow fibers are present in FH 22, FH 77, FH 88 and with a wall thickness of 50 um in FH 66 (Trademark names).

- Polyamide for water ultrafiltration (for the separation of pyrogens) with a wall thickness of 60 um designed as a "finger structure" of lower hydrophilicity. The internal diameter of the hollow fiber is 215 μ m. These hollow fibers are present in U 2000 and U 7000 (Trademark names).

- *The KF 201 EVAL N13 is a ethylene-vinyl alcohol copolymer hollow fiber membrane with a wall thickness of 32 um. This membrane is also present in KF 101 EVAL N16.

- *The TORAY PMMA hollow fiber membrane is an asymmetric membrane on the basis of polymethylmethacrylate with a wall thickness of 30 um in B1-1.6U and B1-2.1U or of 20 um in B2-0.5 up to B2-2.0 and B1-0.6H up to B1-1.6H.

- *The BAXTER CT 100 G is a cellulose triacetate hollow fiber membrane. It has been described as a hybrid cellulose material with a symmetric pore size distribution and a wall thickness of 15 um. This type of hollow fibers is also present in BAXTER CT 190 G.

- Each dialyzer was impregnated before its use by preperfusion with a human serum albumin solution (5 g/100 ml) on the blood side as well as on the dialysate side with a flow rate of 50 ml/min for a period of 20 min.

Abbreviations in the tables:

- | | |
|----------|---|
| Alb. | - Albumin |
| Un. Bil. | - unconjugated Bilirubin |
| c. Bil. | - conjugated Bilirubin |
| SBP | - Sulphobromophtalein |
| Ph | - Phenol |
| FFA | - Free fatty acid, in this case caprylic acid |

DIG - Digitoxin
 Crea - Creatinine
 Ur. acid - Uric acid

5 D. In vitro Results:

a) Evaluation of the Principle

10 Tab.1 Removal of PBS from plasma (liquid A) in vitro (HF 80, Fresenius AG), plasma concentrations (in liquid A):

	Time (min)	Alb. (g/dl)	Un.Bil. (mg/dl)	SBP (mg/l)	Ph (mg/l)	FFA (mg/l)
15	0	3.5	11.70	230	529	749
	5	3.4	9.36	186	370	562
	10	3.4	8.42	151	291	502
	30	3.4	7.72	133	190	434
	60	3.4	7.02	124	137	419
20	90	3.3	5.85	117	127	412

In order to demonstrate the importance of the albumin in the dialysate liquid (B) a comparative test was performed under the same conditions as in Tab. 1 but omitting the albumin in
 25 the dialysate liquid (B). The results are shown in Comparative Tab.1.

40

Comparative Tab.1. Removal of PBS from plasma (liquid A) in vitro (HF 80, Fresenius AG), plasma concentrations (in liquid A) using a conventional dialysate liquid without albumin:

5						
	Time	Alb.	Un.Bil.	SBP	Ph	FFA
	(min)	(g/dl)	(mg/dl)	(mg/l)	(mg/l)	(mg/l)
	0	3.5	11.70	230	529	749
10	5	3.4	11.5	227	420	730
	10	3.4	11.42	221	371	723
	30	3.4	11.44	218	290	719
	60	3.4	11.39	211	270	718
	90	3.3	11.40	215	268	724

15

Tab.2. Removal of PBS from plasma (Liquid A) (HF 80, Fresenius AG), dialysate concentrations (in liquid B):

	Time	Alb.	Un.Bil.	SBP	Ph	FFA
20	(min)	(g/dl)	(mg/dl)	(mg/l)	(mg/l)	(mg/l)
	0	4.0	0.00	0	0	0
	5	3.0	0.9	21	52	149
	10	3.1	1.5	48	79	187
25	30	2.9	1.7	67	100	202
	60	2.9	2.1	103	111	217
	90	2.8	2.3	119	116	224

30 A comparative test was performed under the same conditions as in Tab. 2 but omitting the albumin in the dialysate liquid (B). The results are shown in Comparative Tab.2.

Comparative Tab. 2. Removal of PBS from plasma (Liquid A) (HF 80, Fresenius AG), dialysate concentrations (in liquid B) using a conventional dialysate liquid without albumin:

5	Time (min)	Alb. (g/dl)	Un.Bil. (mg/dl)	SBP (mg/l)	Ph (mg/l)	FFA (mg/l)
	0	0.0	0.0	0	0	0
	5	<0.2	0.0	0	28	0
10	10	<0.2	0.0	0	79	0
	30	<0.2	0.0	0	121	0
	60	<0.2	0.0	0	173	0
	90	<0.2	0.0	0	181	0

15 b) From Different Types of Dialyzers/Membranes

Tab. 3. In vitro removal of PBS and hydrophilic toxins from plasma (liquid A) (Amicon Diafilter), plasma concentrations (liquid A):

20	time (min)	Alb (g/dl)	SBP (mg/l)	Un.Bil (mg/dl)	c.Bil. (mg/dl)	Crea (mg/dl)	Urea (mg/dl)	Ur.acid (mg/dl)
	0	3.5	230	11.7	0.6	6.8	120	16.8
25	30	3.4	86	4.5	0.47	3.2	37	9.8
	60	3.3	56	3.9	0.40	2.9	37	8.1

42.

Tab. 4. In vitro removal of PBS and hydrophilic toxins from plasma (liquid A) (Amicon Diafilter), dialysate concentrations (liquid B):

5	time (min)	Alb (g/dl)	SBP (mg/l)	Un.Bil (mg/dl)	c.Bil. (mg/dl)	Crea (mg/dl)	Urea (mg/dl)	Ur.acid (mg/dl)
	0	4.0	0	0.00	0.00	0.0	0	0.0
	30	3.3	9	0.46	0.14	3.6	47	10.3
10	60	3.3	44	0.91	0.36	3.5	47	10.3

Tab. 5. In vitro removal of PBS and hydrophilic toxins from plasma (liquid A) (FH 88 from GAMBRO), plasma concentrations (liquid A):

15	time (min)	Alb (g/dl)	SBP (mg/l)	Un.Bil (mg/dl)	c.Bil. (mg/dl)	Crea (mg/dl)	Urea (mg/dl)	Ur.acid (mg/dl)
	0	3.5	230	11.7	0.6	6.8	120	16.8
20	30	3.2	47	7.3	0.13	2.8	19	3.0
	60	3.1	32	6.3	0.13	2.7	12	3.0

Tab. 6. In vitro removal of PBS and hydrophilic toxins from plasma (liquid A) (FH 88 from GAMBRO), dialysate concentrations (liquid B):

25	time (min)	Alb (g/dl)	SBP (mg/l)	Un.Bil (mg/dl)	c.Bil. (mg/dl)	Crea (mg/dl)	Urea (mg/dl)	Ur.acid (mg/dl)
30	0	4.0	0	0.00	0.00	0.0	0	0.0
	30	3.5	33	1.08	0.07	3.5	30	6.9
	60	3.3	49	1.68	0.12	3.4	28	6.9

Tab . 7 . In vitro removal of PBS and hydrophilic toxins from plasma (liquid A) (CT 110 G from BAXTER), plasma concentrations (liquid A):

	time (min)	Alb (g/dl)	SBP (mg/l)	Un.Bil (mg/dl)	c.Bil. (mg/dl)	Crea (mg/dl)	Urea (mg/dl)	Ur.acid (mg/dl)
5	0	3.4	232	11.7	0.6	6.8	120	16.8
	30	3.3	114	8.8	0.5	3.0	29	8.2
10	60	3.2	84	7.6	0.45	3.0	38	8.3

Tab. 8. In vitro removal of PBS and hydrophilic toxins from plasma (liquid A) (CT 110 G from BAXTER), dialysate concentrations (liquid B):

	time (min)	Alb (g/dl)	SBP (mg/l)	Un.Bil (mg/dl)	c.Bil. (mg/dl)	Crea (mg/dl)	Urea (mg/dl)	Ur.acid (mg/dl)
15	0	4.0	0	0.00	0.00	0.0	0	0.0
	30	3.5	59	0.8	0.02	3.0	39	8.1
20	60	3.3	84	1.1	0.02	3.0	38	8.1

Tab. 9. In vitro removal of PBS and hydrophilic toxins from plasma (liquid A) (SPAN from Organon Technika), plasma concentrations liquid A):

	time (min)	Alb (g/dl)	SBP (mg/l)	Un.Bil (mg/dl)	c.Bil. (mg/dl)	Crea (mg/dl)	Urea (mg/dl)	Ur.acid (mg/dl)
30	0	3.5	240	11.7	0.6	6.8	120	16.8
	30	3.2	167	9.2	0.58	2.7	38	6
	60	3.2	148	8.6	0.58	2.5	38	6

Tab. 10. In vitro removal of PBS and hydrophilic toxins from plasma (liquid A) (Span von Organon Technika), dialysate concentrations (liquid B):

	time (min)	Alb (g/dl)	SBP (mg/l)	Un.Bil (mg/dl)	c.Bil. (mg/dl)	Crea (mg/dl)	Urea (mg/dl)	Ur.acid (mg/dl)
5	0	4.0	0	0.00	0.00	0.0	0	0.0
	30	3.6	6	0.19	0.17	2.6	38	7.0
10	60	3.6	18	0.19	0.18	2.6	38	7.0

c) Influence of Different Albumin Concentrations in the Dialysate Liquid (B)

- Phenol-enriched plasma: Human heparinized plasma was taken from young male donors and enriched with phenol. This was performed by dissolving 530 mg phenol in 50 ml of a 0.1 M NaOH solution. Thereafter 2.5 g human albumin (fatty acid free, SIGMA) were added. Thereafter the pH value was adjusted to 7.4 with a 30 percentage acetate solution.
- Finally this toxin cocktail was mixed with 950 ml pooled plasma from healthy young male donors. 500 ml of this plasma solution were filled into the plasma bottle on the plasma side of a dialyzer.
- Dialysate liquid (B): 200 ml of a 20 percent (20 g/100 ml) human albumin solution were mixed with 300 ml of a commercial CVVH dialysis solution. 500 ml of this solution (i.e. 80 g/l albumin) were filled into the dialysate bottle on the dialysate side of a dialyzer.
- After filling the bottles they were closed hermetically and the pump segments (inflow and outflow) installed to the simultane roller pumps in a manner enabeling recirculation

of each (plasma and dialysate) circle counter currently. The flow rates were adjusted to 100 ml/min. The temperature was adjusted to 37°C.

- 5 Tab. 11. Removal of phenol from plasma (liquid A) in vitro (HF 80, FRESSENIUS AG) with enhanced albumin concentration in the dialysate (8 g/dl), plasma and dialysate concentrations:

10	time	Phenol concentration (mg/l)	
	(min)	liquid A (plasma)	liquid B (dialysate)
	0.0	529	0
	2.5	158	148
	5.0	132	151
15	10.0	116	148
	20.0	105	147

E. In vivo System

20

A commercial dialysis machine (A 2008, Fresenius) was chosen as hardware equipment for the in vivo system. Every single piece was commercially available and had safety approval from the German Federal Health Authority, Berlin, for usage
 25 in a extracorporeal therapeutic blood treatment.

The blood pump of the A 2008 was used to supply continuous blood flow of 160 ml/min through an asymmetric polysulfone hollow fiber dialyzer (1.8 sqm, HF 80, Fresenius), that was
 30 on both sides albumin impregnated as described above. The liquid (B) dialysate compartment was a closed loop system which contained 1000 ml of Ringers lactate solution with albumin in a concentration of 5 g/100ml (i.e. a concentration used for intravenous infusions or liquid

replacement in plasma exchange). The flow rate of this albumin containing liquid (B) dialysate of 120 ml/min was realized by the second pump of the machine (normally used for substitution liquids). Moreover, the in in vitro-system
5 was modified by introducing a three-step regeneration of the albumin-containing liquid (B) dialysate, thus increasing the PBS-separation capacity and adding the possibility of effective hemofiltration and separation of water soluble toxins.

10

The liquid (B) dialysate loop contained in this order:

1. An additional conventional large pore dialyzer (1.3 sqm polysulfon hollow fiber dialyzer, HF 60, FRESSENIUS AG) which
15 was connected to the normal dialysate compartment of the A 2008. While the albumin-containing closed-loop liquid (B) dialysate was passed through the blood compartment of the dialyzer, a standard bicarbonate dialysate (potassium ions 4 mmol/liter) was employed on the other side. The aim of
20 this first step was to remove water-soluble factors of hepatic coma (ammonia, imbalanced amino acids, conjugated bilirubin etc.) to support electrolyte-, glucose- and pH-regulation and to support or replace kidney function in hepatorenal syndrome or other types of kidney failure.

25

2. A commercial column of activated charcoal (Adsorba 300C, GAMBRO AB). The aim of this second step was to remove a first group of PBS (e.g. aromatics, fatty acids) from the albumin in order to facilitate the reuse of the albumin
30 solution in the closed-loop system.

3. A commercial anion exchange resin (Plasorba BR-350, Asahi Medical). The aim of this third step was the separation of unconjugated bilirubin and accumulated bile acids from the
35 albumin solution.

After this regeneration the albumin solution was recirculated into the liquid (B) dialysate compartment of the HF 80 dialyzer for further blood purification.

5 F. In vivo Results

*Patient 1

The patient was a 30 year old caucasian woman with an acute
10 decompensation of a chronic liver failure after a six year
history of alcoholism. The patient was treated for 10 days
conservatively without success. At entry to the treatment
the patient was in grade IV hepatic encephalopathy (i.e.
deep coma, patient not reacting to painful stimuli).
15 Hypotension, hypoglycemia and alcalosis were present.

Biochemistry: thromboplastine time (Quick) 19 %, activated
coagulation time was higher than 200 seconds, antithrombine
III was 18%, platelet count was 73×10^9 /liter (with an
20 decreasing tendency). The total bilirubin was 615
 $\mu\text{mol/liter}$, unconjugated bilirubin 51 $\mu\text{mol/liter}$,
cholinesterase 16 $\mu\text{mol/liter}$, ammonia 96 $\mu\text{mol/liter}$. The
amount of aminotransferases was five to tenfold increased,
the blood pH was 7.5. The patient underwent three treatment
25 procedures (on three subsequent days) with treatment times
from about 7 and ten hours per day using a detoxification
system as described below.

No adverse reactions were observed during the treatment.
30 Blood pressure, oxygen saturation, blood glucose level and
blood pH improved slowly but continuously.

During the treatment the patient recovered from
unconsciousness and fully awoke after the second treatment

without remaining neurological symptoms of encephalopathy (no flapping tremor, fully orientated, no delay in communicational and physical response, recovery of normal reflexes). During the following days the activity of liver enzymes decreased slowly to 50%, the thromboplastine time increased to 40%. Within three following days the platelet count increased up to 130×10^9 /liter and antithrombine III to 43%.

10 Tab.12. Patient 1: Total serum bilirubin levels pre- and post-treatment

15	No. of treatments	time of treatment (h)	Total serum bilirubin		Total decrease (mg/dl)
			before treatment (mg/dl)	after treatment (mg/dl)	
	1	7	36	25.3	10.7
	2	10	34.5	23.2	11.3
20	3	8	23.5	17.5	6.1

Tab.13. Patient 1: Unconjugated serum bilirubin levels pre- and post-treatment

25	No. of treatments	time of treatment (h)	Total serum bilirubin		Total decrease (mg/dl)
			before treatment (mg/dl)	after treatment (mg/dl)	
	1	7	3.0	2.5	0.5
	2	10	3.3	2.3	1.0
30	3	8	1.8	1.7	0.1

*Patient 2 male, 34 years old , chronic alcoholic liver disease

The patient (120kg bodyweight) was known as alcoholic for
5 six years and admitted to the hospital for slowly increasing
jaundice accompanied by signs of infection (fever, high
white blood cell counts, left shift), loss of appetite and
impairment of general status. He was treated for three weeks
conservatively. As the general status worsened rapidly under
10 conservative treatment and serum bilirubin concentrations
rose to levels up to 37.7 mg/100 ml the patient was treated
with the MARS method. During five days of treatment no
further worsening of the general status of the patient did
occur. Treatment time of four hours obviously was too short
15 for the patient. On day 4 a short 2 hour MARS treatment was
combined with a two hours hemodiafiltration. The very low
total bilirubin difference of that day is probably a
combined effect of ineffective treatment and sufficient
hemofiltration. However, even the short treatment time
20 allowed bilirubin separation as well as separation of
hydrophilic uremic toxins like urea, creatinine or ammonia
(due to the second dialyzer enclosed in the dialysate
circuit).

50

Tab. 14. Patient 2: Total bilirubin levels pre- and post-treatment

5	No. of	time of	Total serum bilirubin		Total
	treatments	treatment	before	after	decrease
		(h)	treatment		
			(mg/dl)	(mg/dl)	(mg/dl)
10	1	4	37.7	32.2	5.5
	2	5	37.0	29.1	7.9
	3	4	32.4	27.5	4.9
	4	2+2	32.8	32.1	0.7
	(MARS+HDF)				
	5	4	32.4	28.5	3.9

Tab. 15. Patient 2: Serum urea and creatinine levels preand post-treatment

20	No	time	Urea (mg/dl)		Creatinine	
	of	of	before	after	before	after
	treatments		treatment		treatment	
25	1	4	160	138	3.9	3.1
	2	5	194	153	4.1	3.4
	3	4	208	152	4.7	3.6
	4	2+2	226	136	5.2	3.1
	(Mars+HDF)					
	5	4	202	148	4.9	3.7

*Patient 3 female, 29 years old, chronic alcoholic liver insufficiency

- 5 The patient had a long history of alcohol abuse with a known chronic liver insufficiency that was clinically estimated to be on a precirrhotic level. Admittance happened upon increasing jaundice and worsening of the general status. Under conventional treatment the patient went into hepatic
- 10 encephalopathy grade IV, i.e. she fell into deep coma and did not respond to painful stimuli. Bilirubin levels rose to 29,5 mg/100 ml. She showed life-threatening severe acid-base disturbances, pulmonary edema, hypoxemia on the first day of MARS-treatment and was estimated moribund by experienced
- 15 medical doctors. In this status treatment with the method according to the present invention was started as ultima ratio. Within two days the severe life-threatening status totally changed into a moderate state of disease with normal acid-base values, normooxygenation and normal ventilation.
- 20 The patient awoke from coma and went back to encephalopathy grade I/II, i.e. she reacted adequately although with a slurred speech. The patient was treated for six consecutive days for six hours per day. The bilirubin level continuously decreased to 16,2 mg/100 ml during this time.

Tab. 16. Patient 3: Total serum bilirubin levels pre- and post-treatment during 4 MARS sessions (1-4) and two hemodiafiltration sessions (5-6)

5	No. of treatments	time of treatment (h)	Total serum bilirubin		Total decrease (mg/dl)
			before (mg/dl)	after treatment (mg/dl)	
10	1	5	29.0	24.3	4.7
	2	6	26.9	19.9	7.0
	3	5	23.4	17.8	5.6
	4	5	18.3	13.1	5.2
	5	5	15.9	12.9	3.0
15	6	4.5	16.5	15.6	0.9

*Patient 4

female, 41 years old, digoxin intoxication

- 20 The patient showed typical clinical signs of digoxin intoxication such as tachyarrhythmia, loss of appetite, vomiting, diarrhoea; ECG showed ventricular arrhythmia and typical ST-changes. After four hours of treatment the clinical symptoms normalized completely and so did the ECG changes.
- 25

Tab. 16 shows single pass clearances at the start and at the end of the treatment. A highly toxic starting level and sufficient regeneration of digoxin by on line-adsorption/dialysis in the closed-loop liquid (B) dialysate circuit (3,95 to 0,5 and 2,55 to 0,54) were noted. No saturation of the liquid (B) dialysate circuit and the adsorbents (PBS) was recognized. Since digoxin is distributed in many tissues the blood level normally

30

decreases very slowly because of continuous influx from the other tissues. Therefore a longer treatment, ideally a continuous one, would be desirable.

5 Tab.17. Patient 4: Single pass clearances of digoxin during 4 hours of MARS

time	location	digoxin	
		serum	dialysate
		concentration ($\mu\text{mol/l}$)	
10	start	pre	
		dialyzer	
		7.45	0.50
15		post	
		dialyzer	
		3.66	3.95
	end	pre	
		dialyzer	
		5.84	0.54
20		post	
		dialyzer	
		3.86	2.55

The above is intended to illustrate the present invention but is not limiting. Numerous variations and modifications can be effected without departing from the spirit and scope of the novel concepts of the invention. It is to be understood that no limitation with respect to the specific compositions and uses described herein is intended or should be inferred.

It is to be observed that even if the present invention relates to for albumin impermeable or essentially impermeable membranes, it includes also the use of membranes partly permeable for albumin.

CLAIMS

- 5 1. Membrane for the separation of protein-bound
substances from a protein-containing liquid (A) containing
these substances by dialysis against a dialysate liquid
(B), wherein a protein having an acceptor function for the
protein-bound sub-stances is attached to at least one side
10 of the membrane and the membrane has such a pore size that
the protein-bound substances can pass through the membrane.
2. Membrane according to claim 1 comprising two
functionally different parts (regions), one part having an
actual separating membrane function permitting the protein-
15 bound sub-stances to pass through and excluding the pro-
tein(s) which had bound the protein-bound substances in
liquid (A) and the acceptor protein in liquid (B), and the
other part having a port- and adsorption-function, and the
membrane being coated on at least one side with a protein
20 having an acceptor functions for the protein-bound sub-
stances.
3. Membrane according to claim 1 or 2, comprising
one part having an actual separating membrane function with
a tunnel-like structure in the liquid (A) side, the tunnels
25 having a length less than about 10 μm and having a diameter
sufficiently small to exclude the protein in liquid (A) and
the acceptor protein in liquid (B), and a part with a port-
and adsorption-structure on the dialysate liquid (B) side.
4. Membrane according to any of the claims 1-3,
30 wherein the length of the tunnels is less than about 5 μm .
5. Membrane according to any of the claims 1-3,
wherein the length of the tunnels is less than about 0.1
 μm .

6. Membrane according to any of the claims 1-3, wherein the membrane material is selected from the group consisting of polysulfones, polyamides, polycarbonates, 5 polyesters, acrylonitrile polymers, vinyl alcohol polymers, acrylate polymers, methacrylate polymers, and cellulose acetate polymers.

7. Membrane according to any of the claims 1-3, wherein the membrane material is a polysulfone.

10 8. Membrane according to claim 1 or 2, wherein the membrane material is a polysulfone.

9. Method for the separation of protein-bound substances from a protein-containing liquid (A) containing these substances comprising dialyzing said liquid (A) 15 against a dialysate liquid (B) by means of a membrane, said membrane permitting passage of the protein-bound substances to the dialysate liquid (B) side, and by means of a protein having an acceptor function for the protein-bound substances, and said acceptor protein being present either in 20 free form in the dialysate liquid (B) and/or attached to at least one side of the membrane.

10. Method for the separation of protein-bound substances from a protein containing liquid (A) containing these substances comprising dialyzing said liquid (A) 25 against dialysate liquid (B) containing a protein having an acceptor function for the protein-bound substances and by means of a membrane comprising two functionally different parts, one part having an actual separating membrane function permitting passage of the protein-bound substances and 30 the water-soluble substances and excluding the protein(s) which had bound the protein-bound substances in liquid (A) and the acceptor protein in liquid (B), and the other part having a port- and adsorption-function, and the membrane being coated with a protein having an acceptor function for 35 the protein-bound substances.

11. Method according to claim 9 or 10, wherein the membrane comprises one part having an actual separating membrane function with a tunnel-like structure on the liquid (A) side, the tunnels having a length less than about 10 μm and having a diameter sufficiently small to exclude the protein in liquid (A) and the acceptor protein in liquid (B), and a part with a port- and adsorption-structure on the dialysate liquid (B) side.
12. The method of claim 11, wherein the length of the tunnels of the membrane is less than about 5 μm .
13. The method of claim 11, wherein the length of the tunnels of the membrane is less than about 0.1 μm .
14. The method of claim 11, wherein the membrane material is selected from the group consisting of polysulfones, polyamides, polycarbonates, polyesters, acrylonitrile polymers, vinyl alcohol polymers, acrylate polymers, methacrylate polymers, and cellulose acetate polymers.
15. The method of claim 14, wherein the membrane material is a polysulfone.
16. The method of claim 9, 10 or 11, wherein the protein-containing liquid (A) is selected from the group consisting of plasma and blood and the dialysate liquid (B) comprises human serum albumin as a protein having an acceptor function for the protein-bound substances.
17. The method of claim 9, 10 or 11, wherein the membrane is coated with a solution comprising a protein having an acceptor function for the protein-bound substances.
18. The method of claim 17, wherein the membrane is coated with a solution comprising human serum albumin as a protein having an acceptor function for the protein-bound substances.

19. The method of claim 9 or 10, wherein the dialysate liquid (B) comprises human serum albumin in a concentration from about 1 to about 50 grams per 100 ml.

5 20. The method of claim 9 or 10, wherein the dialysate liquid (B) comprises human serum albumin in a concentration from about 6 to about 40 grams per 100 ml.

21. The method of claim 9 or 10, wherein the dialysate liquid (B) comprises human serum albumin in a
10 concentration from about 8 to about 30 grams per 100 ml.

22. The method of claim 9 or 10, wherein the dialysate liquid (B) comprises human serum albumin in a concentration from about 8 to about 20 grams per 100 ml.

23. A disposable set for the separation of pro-
15 tein-bound substances from plasma or blood containing these substances including a dialyser comprising a membrane according to any one of claims 1 to 8.

24. A disposable set according to claim 23,
wherein the dialyzer contains on the dialysate liquid (B)
20 side a human serum albumin containing liquid.

25 25. A disposable set for the separation of protein-bound substances from plasma or blood containing these substances including dialyzer comprising a membrane according to any one of claims 1 to 8, a second conventional dialyzer for hemodialysis, and preferably a conventional charcoal adsorber unit for hemoperfusion and a conventional ion exchange resin unit for hemoperfusion interconnected by tubing and a unit of a human serum albumin containing dialysate liquid (B).

26. A disposable set for the separation of protein-bound substances from plasma or blood containing said substances including a dialyzer comprising a membrane according to any one of claims 1 to 8 and being filled on
5 the dialysate liquid (B) side with a human serum albumin containing liquid, a second conventional dialyzer for hemodialysis, a conventional charcoal adsorber unit for hemoperfusion, and preferably a conventional ion exchange resin unit for hemoperfusion interconnected by tubing and a
10 unit of a human serum albumin containing dialysate liquid.

27. A dialysate liquid (B) that is bicarbonate buffered comprising in form of ions sodium from about 130 to about 145 mmol/1000 ml, calcium from about 1.0 to about 2.5 mmol/1000 ml, potassium from about 2.0 to about 4.0
15 mmol/1000 ml, magnesium from about 0.2 to about 0.8 mmol/-1000 ml, chloride from about 100 to about 110 mmol/1000 ml, acetate from about 2 to about 10 mmol/1000 ml, and human serum albumin from about 1 to about 50 g/100 ml.

28. A dialysate liquid (B) that is bicarbonate buffered comprising in form of ions sodium from about 130 to about 145 mmol/1000 ml, calcium from about 1.0 to about 2.5 mmol/1000 ml, potassium from about 2.0 to about 4.0
20 mmol/1000 ml, magnesium from about 0.2 to about 0.8 mmol/-1000 ml, chloride from about 100 to about 110 mmol/1000 ml, bicarbonate from about 30 to about 40 mmol/1000 ml, acetate
25 from about 2 to about 10 mmol/1000 ml, and human serum albumin from about 6 to about 40 g/100 ml.

29. A dialysate liquid (B) that is bicarbonate buffered comprising in form of ions sodium from about 130 to about 145 mmol/1000 ml, calcium from about 1.0 to about 2.5 mmol/1000 ml, potassium from about 2.0 to about 4.0
30 mmol/1000 ml, magnesium from about 0.2 to about 0.8 mmol/-1000 ml, chloride from about 100 to about 110 mmol/1000 ml, bicarbonate from about 30 to about 40 mmol/1000 ml, acetate
35 from about 2 to about 10 mmol/1000 ml, and human serum albumin from about 8 to about 30 g/100 ml.

30. A dialysate liquid (B) that is bicarbonate buffered comprising in form of ions sodium from about 130 to about 145 mmol/1000 ml, calcium from about 1.0 to about 2.5 mmol/1000 ml, potassium from about 2.0 to about 4.0 mmol/1000 ml, magnesium from about 0.2 to about 0.8 mmol/1000 ml, chloride from about 100 to about 110 mmol/1000 ml, bicarbonate from about 30 to about 40 mmol/1000 ml, acetate from about 2 to about 10 mmol/1000 ml, and human serum albumin from about 8 to about 20 g/100 ml.

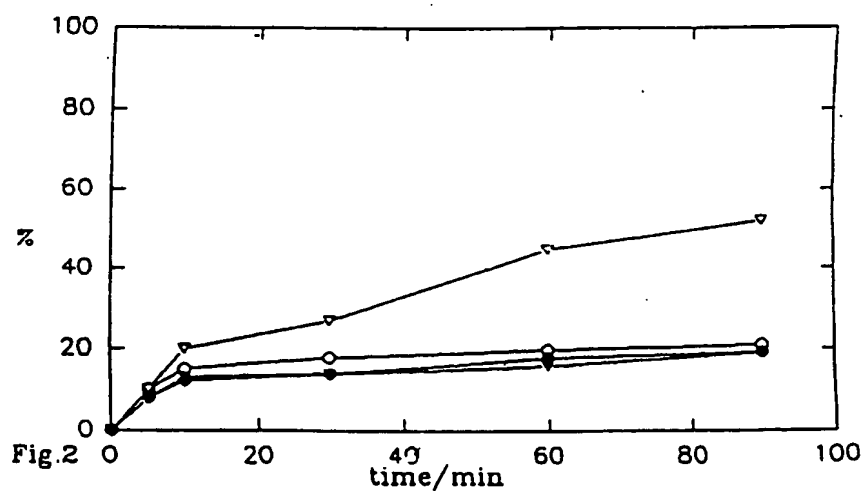
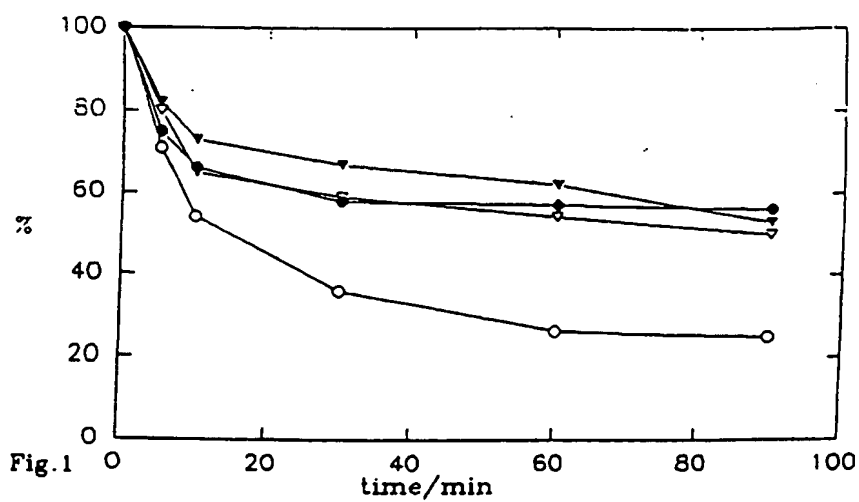
31. A dialysate liquid (B) that is acetate buffered comprising in form of ions sodium from about 130 to about 145 mmol/1000 ml, calcium from about 1.0 to about 2.5 mmol/1000 ml, potassium from about 2.0 to about 4.0 mmol/1000 ml, magnesium from about 0.2 to about 0.8 mmol/1000 ml, chloride from about 100 to about 110 mmol/1000 ml, acetate from about 30 to about 40 mmol/1000 ml, human serum albumin from about 1 to about 50 g/100 ml.

32. A dialysate liquid (B) that is acetate buffered comprising in form of ions sodium from about 130 to about 145 mmol/1000 ml, calcium from about 1.0 to about 2.5 mmol/1000 ml, potassium from about 2.0 to about 4.0 mmol/1000 ml, magnesium from about 0.2 to about 0.8 mmol/1000 ml, chloride from about 100 to about 110 mmol/1000 ml, acetate from about 30 to about 40 mmol/1000 ml, human serum albumine from about 6 to about 40 g/100 ml.

33. A dialysate liquid (B) that is acetate buffered comprising in form of ions sodium from about 130 to about 145 mmol/1000 ml calcium from about 1.0 to about 2.5 mmol/1000 ml, potassium from about 2.0 to about 4.0 mmol/1000 ml, magnesium from about 0.2 to about 0.8 mmol/1000 ml, chloride from about 100 to about 110 mmol/1000 ml, acetate from about 30 to about 40 mmol/1000 ml, human serum albumin from about 8 to about 30 g/100 ml.

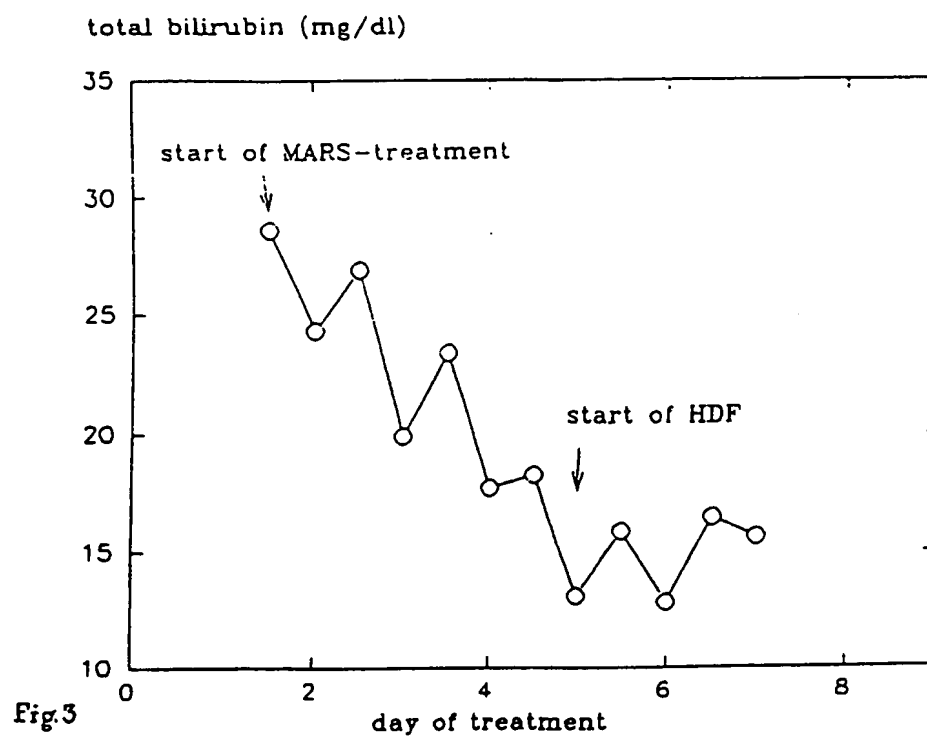
34. A dialysate liquid (B) that is acetate buffered comprising in form of ions sodium from about 130 to about 145 mmol/1000 ml, calcium from about 1.0 to about 2.5
5 mmol/1000 ml, potassium from about 2.0 to about 4.0 mmol/-1000 ml, magnesium from about 0.2 to about 0.8 mmol/1000 ml, chloride from about 100 to about 110 mmol/1000 ml, acetate from about 30 to about 40 mmol/1000 ml, human serum albumin from about 8 to about 20 g/100 ml.

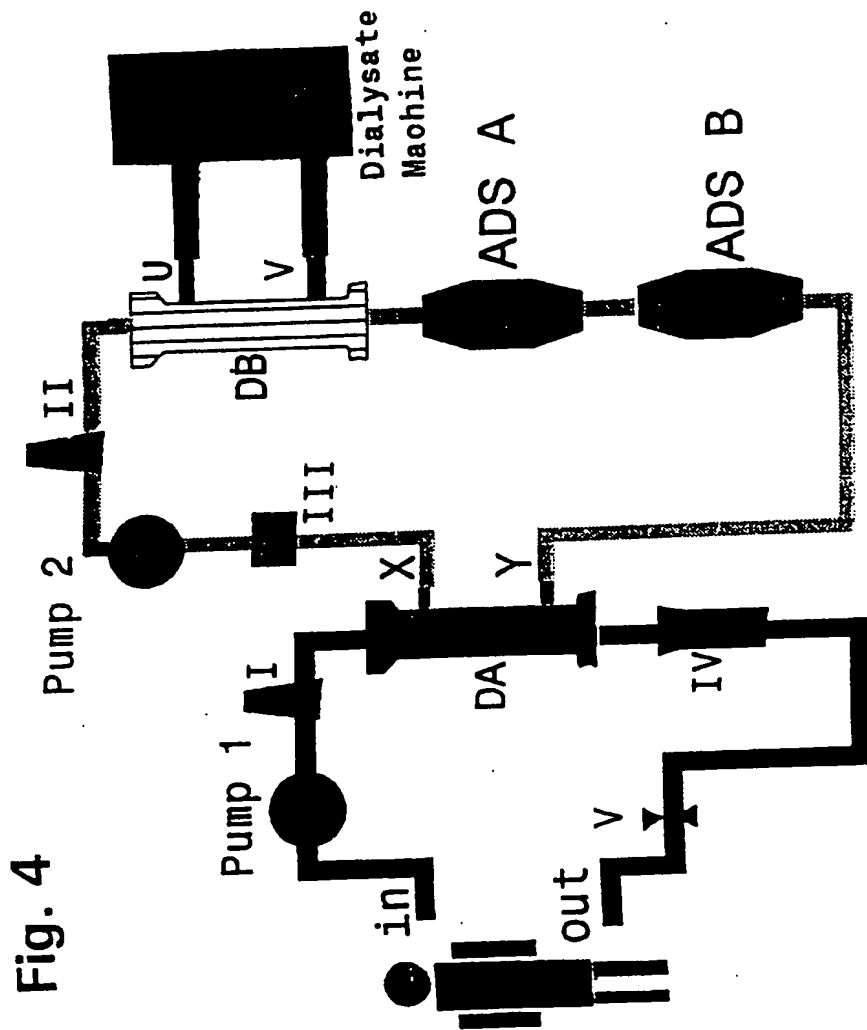
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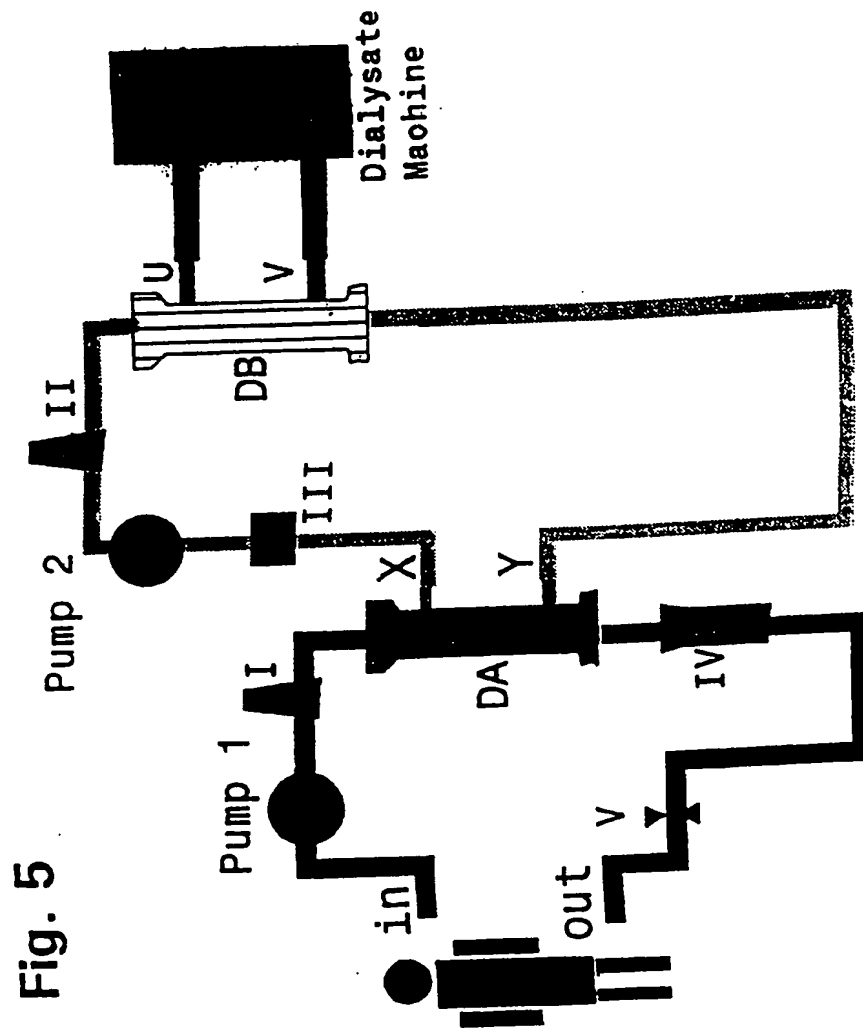
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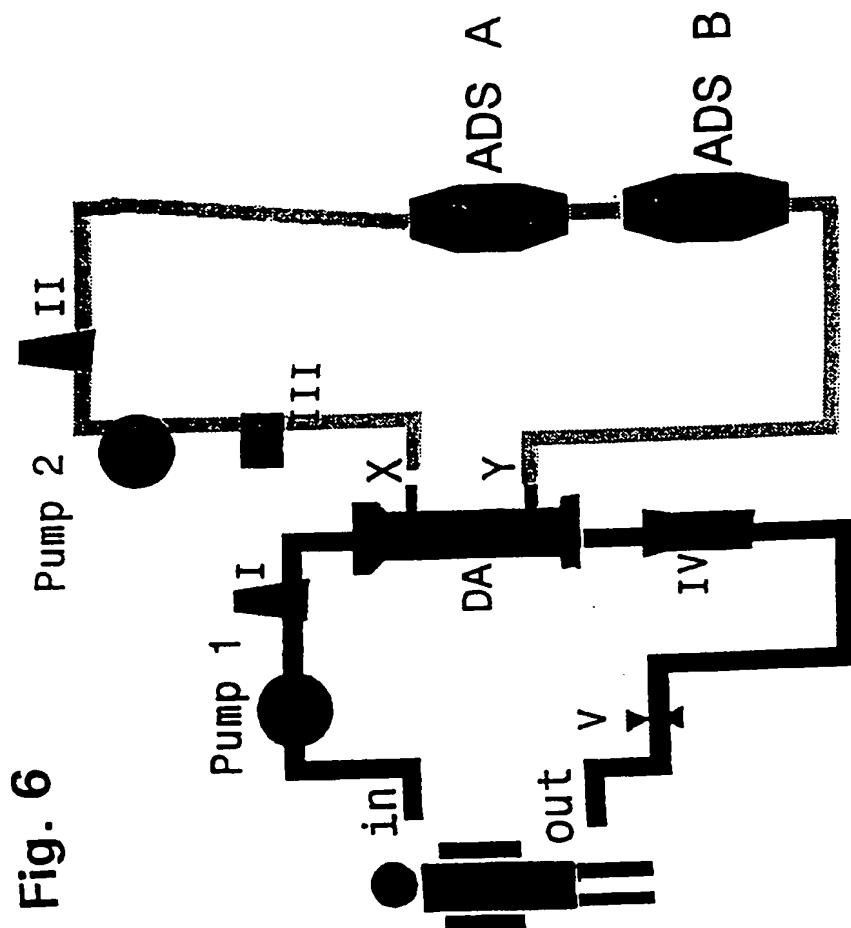
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 94/00073

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 B01D69/14 B01D61/24 A61M1/34

According to International Patent Classification (IPC) or to both national classification and IPC.

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 B01B B01D A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 102 564 (FRAUNHOFER-GESELLSCHAFT ZUR FÖRDERUNG DER ANGEWANDTEN FORSCHUNG E. V.) 14 March 1984 see the whole document ---	1
A	US,A,5 078 885 (K. N. MATSUMURA) 7 January 1992 see the whole document ---	1,9
A	WO,A,91 12072 (CREATIVE BIOMOLECULES, INC) 22 August 1991 ---	
A	EP,A,0 168 140 (THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK) 15 January 1986 -----	

☐ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

13 July 1994

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 94/00073

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